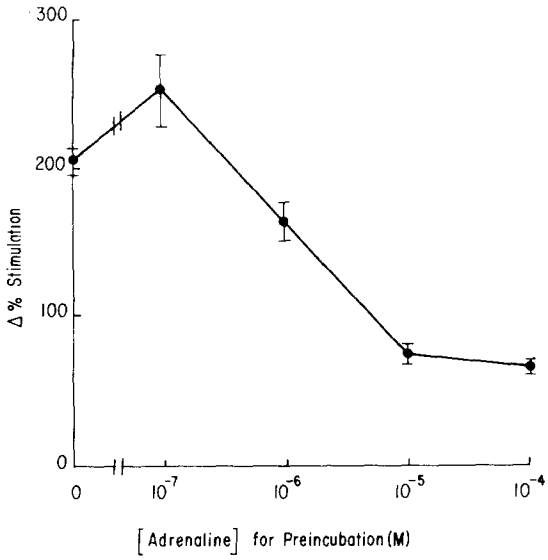

Fig. 1. Dose dependence of desensitization. Slices were preincubated for 1 hr at various adrenaline concentrations prior to preparation of adenylate cyclase. Each point represents the mean ± S.E.M. of three batches of slices (new-born rats).

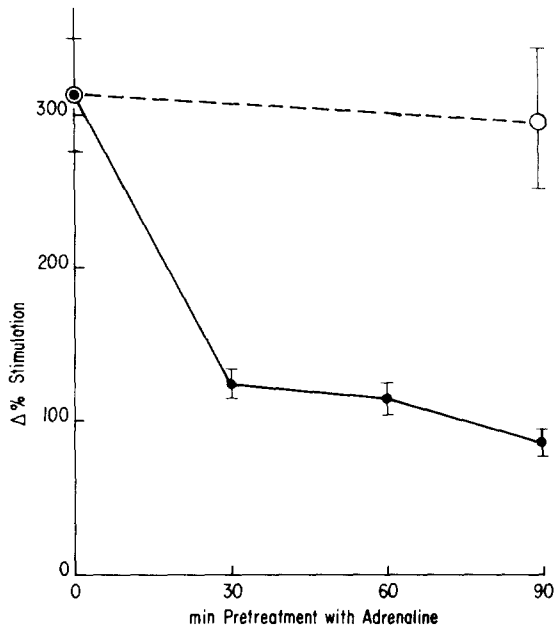

Fig. 2. Time dependence of desensitization. For each point three batches of slices (new-born rats) were incubated with 10^{-5} M adrenaline for various times prior to preparation of adenylate cyclase. One point (○) represents a control incubated without adrenaline for 90 min. Means ± S.E.M.

A loss of the β-adrenergic receptor binding function has been noted in catecholamine-treated frog erythrocytes [5]. It is possible that a cAMP-dependent phosphorylation reaction on the level of adenylate cyclase or the hormone receptor is involved.

The significance of the present desensitization phenomenon remains to be investigated. The chronic treatment of rats with adrenaline was found to lead to a suppression of the adrenaline-induced glycolytic response [9]. It is possible that this pharmacological effect is based on the desensitization of liver adenylate cyclase. It should be noted that the concentrations of adrenaline used in the present study were considerably higher than those found *in vivo*, and this fact should be taken into account when considering a possible physiological relevance of our data.

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* Recipient of a STEP summer stipend by the Alberta Department of Secondary Education and Manpower.

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Non-specificity of sulphydryl inhibition of the alpha adrenergic response

(*Received* 6 *March* 1976; *accepted* 7 *April* 1976)

Several reactive chemical groups have been suggested as possible binding sites for agonists and antagonists on the alpha adrenergic receptor [1–5]. Prominent amongst these suggestions is that of the involvement of the sulphydryl group [1, 6, 7].

Protein has been suggested as the foundation material for the structure of the alpha adrenergic receptor [8, 9], and irreversible alpha receptor blocking agents have been shown to interact with protein and its constituents [9–11]. In view of the relationship between free sulphydryl groups

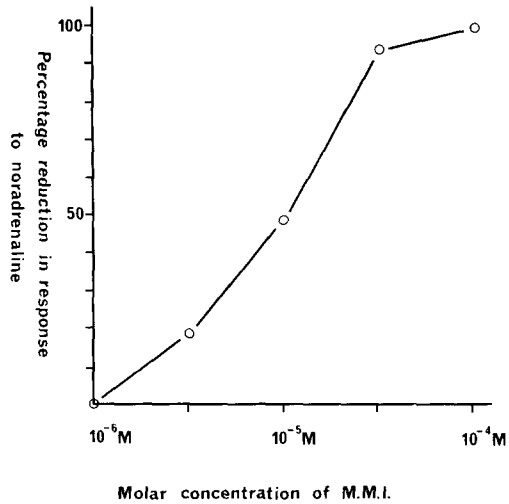

Fig. 1. Graph showing the dose–percentage response relationship for the inhibitory action of methyl mercuric iodide (M.M.I.) against standard responses to the agonist noradrenaline, in guinea pig vas deferens. Each point represents the mean of 4 experiments.

and protein, the possibiltiy of both being involved in the make-up of the alpha receptor cannot be overlooked.

It was therefore considered important to clarify the position as to whether or not sulphydryl groups play an integral part in the make-up of the alpha adrenergic receptor site.

Methods

Paired vasa from 350–400 g guinea pigs were suspended in 10 ml of Hukovic solution [12] at 37° and gassed with 5% carbon dioxide in oxygen.

Standard responses to the agonists, noradrenaline, acetylcholine and histamine were obtained prior to the addition of varying doses of the sulphydryl inhibitor, methyl mercuric iodide (M.M.I.). Following 10-min. exposure to M.M.I., responses to the agonists were again recorded. Percentage reductions in responses to the agonists were calculated. Drugs used: noradrenaline acid tartrate; acetylcholine chloride; histamine diphosphate; methyl mercuric iodide.

Results and Discussion

The sulphydryl inhibitor, M.M.I., reduced the responses to noradrenaline in guinea pig vas deferens, a tissue rich in sympathetic innervation [13, 14]. This reduction was dose-dependent, the ED_{50} for M.M.I. being 10^{-5} M, whilst a dose of 10^{-4} M M.M.I. produced complete inhibition of standard responses to noradrenaline (Fig. 1). The inhibitory effect of M.M.I. was reversible, the responses to noradrenaline returning to pre-inhibition levels within 30 min. following repeated washes with Hukovic's solution at 5-min intervals.

To determine the specificity of inhibitory action of M.M.I., selected dose levels were used against standard responses of the agonists, acetylcholine and histamine in guinea pig vasa. Results are shown in Table 1. The ED_{50} dose of M.M.I. against noradrenaline produced similar inhibitory effects against acetylcholine and histamine.

Table 1. Comparison of the inhibitory effects of M.M.I. against the contractions produced by noradrenaline, acetylcholine and histamine in guinea pig vas deferens

Agonist and dose	Percentage reduction in response to agonist following M.M.I.	
	10^{-5} M	10^{-4} M
Noradrenaline 7×10^{-7} M	49 ± 2.0	99 ± 0.7
Acetylcholine 1×10^{-6} M	65 ± 2.3	98 ± 1.0
Histamine 1.7×10^{-6} M	43 ± 2.1	100 ± 0.5

Equally, a 10^{-4} M dose of M.M.I. produced maximal inhibitory effects against all three agonists.

Results are therefore in accord with those of Goldman and Hadley [7], in that sulphydryl inhibition effectively reduces the noradrenergic response, mediated via alpha receptors. However the results of the present study indicate that this antagonistic response against noradrenaline is not specific. M.M.I. is equipotent in reducing responses to all three agonists, noradrenaline, acetylcholine and histamine. No documented evidence has been reported to link the molecular structures of the receptor sites for these three agonists, therefore, contrary to the hypotheses of D'Iorio and Lague [6], and Goldman and Hadley [7], it is suggested that sulphydryl groups play no part in the structural conformation of the alpha receptor site, but that sulphydryl inhibition is a non-receptor mediated phenomenon, probably associated with the contractile process "beyond" the primary drug-receptor binding site.

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